Improving Trout Through Genetics Research

The ARS National Center for Cool and Cold Water Aquaculture has been busy since its start-up in August 2001. The tank/aquarium part of this new facility in Leetown, West Virginia, now holds 145 families of rainbow trout *(Oncorhynchus mykiss).* "These fish are lending their DNA for genetic analysis, and some are being grown at other research locations to determine how they perform under varying production conditions," says center director William K. Hershberger.

The center's research priorities include fish genetics and breeding, aquatic animal health, nutrition, production system development, and environmental compatibility. Initial research focuses on rainbow trout and other salmonids, but later research could include species such as striped bass, walleye, and yellow perch.

Fish and Chips

The first generation of breeder fish, formed by cross-breeding among two commercially used strains, is complete, says Hershberger. There are now 2,500 young fish from the first set of crosses at the center. They weigh an average of almost 2 pounds, and each has a computer chip embedded for individual identification.

Siblings of the breeder fish were shipped to other locations so their performance could be evaluated under different conditions. Some are being raised at the University of Idaho's Hagerman Fish Culture Experiment Station as part of the cooperative research program. Beyond evaluating growth and other performance traits in a different set of environmental conditions, the Idaho laboratory will also test the fish on different diets. The goal is to develop feeds that have more sustainable ingredients that are used more efficiently and allow the optimum expression of desired traits, such as rapid growth. (See article below.)

Other fish from the same family are being raised in a program with West Virginia University to evaluate their performance in small production unit conditions.

The center includes a 20,000-square-foot aquarium building with the latest in water-treatment and recirculation technology, much of which was developed from research conducted at the Conservation Fund's Freshwater Institute in Shepherdstown, West Virginia—another cooperator in the center's program.

Superb Trout—It's a Matter of Muscle

Some fans of rainbow trout have dubbed their favorite fish "nature's fast food." That's because it takes only a few minutes to pan-fry, grill, or microwave this tasty fish.

Trout's tender, delicately flavored meat is, of course, muscle. Farm-raised trout put on muscle as they swim against the gentle, steady current that runs through the tanks, ponds, or channel-like raceways they grow up in.

New studies should help researchers and rainbow trout breeders more easily single out fish that have the genetic makeup to develop more muscle more quickly. Agricultural Research Service fish geneticist Kenneth E. Overturf is doing this work with ARS and University of Idaho colleagues.

Of particular interest: trout that muscle up rapidly on environmentally friendly, grain-based feeds, made from oats or barley, for instance.

Why the need for faster-growing fish with a hearty appetite for grain?

First, to meet tomorrow's demand for farm-raised fish. Projections indicate that U.S. aquaculture production will have to increase by 500 percent over the next 25 years to satisfy America's needs. Second, to lessen the need to harvest ocean fish, such as menhaden or jack mackerel. Today, these species are key ingredients in feeds for their on-farm cousins.

Overturf, who is with ARS' Idaho-based Small Grains and Potato Research Unit, has developed a high-tech test that sizes up a key aspect of a trout's growth. The test correlates the presence of a telltale protein—myosin—to muscle growth. Results give users a valuable new way to pinpoint trout that have, in their genetic makeup, a superior ability to convert feed into muscle.

He did the studies with rainbow trout at the University of Idaho's Hagerman

STEPHEN AUSMUS (K10424-1)

Geneticist Ken Overturf collects immature trout, or fingerlings, in the wet lab for weight and other measurements to determine how the fish use different feeds.

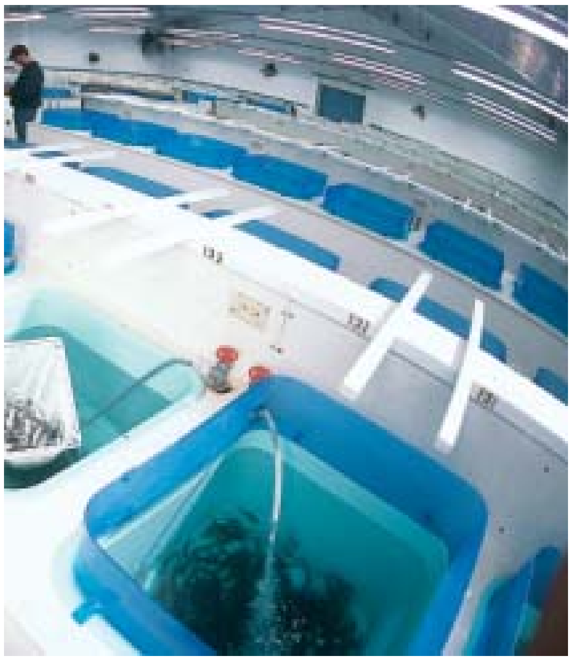
STEPHEN AUSMUS (K10421-1)

Measuring just under half an inch, a Personal Identification Tag, or PIT (the small red device on the right) can be inserted under the skin of a fish for accurate identification.

The DNA Trail

Rainbow trout is one of the major U.S. fish crops. But there has not been much use of genetically based technologies to improve production efficiency in this species. ARS researchers are working hard to glean information from rainbow trout DNA that will be used to find out which fish grow faster, are more resistant to disease, or tolerate stress better. The first order of business is to identify fish that exhibit the desired traits based on family history. For instance, tracking the growth rates of fish can show which are the fastest growers. After individuals are identified with the desired traits, it has to be determined that these traits are indeed passed from parents to offspring. This is accomplished by using designed crosses and statistical analyses. Only after it has been confirmed can DNA analysis begin.

Molecular biologist Caird E. Rexroad III is working on a genetic map of *O. mykiss* that will assist in development of improved strains of the fish for aquaculture. To produce a genetic map, researchers collect blood or tissue samples from trout family members in which a certain trait is prevalent. Using



Fish Culture Experiment Station, about 90 miles southeast of Boise.

Overturf's test provides reliable results very quickly—and doesn't harm the fish. It can be performed at a research laboratory or at any commercial lab equipped to run leading-edge tests known as real-time polymerase chain reaction (PCR) assays.

The test could identify the best-performing rainbow trout as prospective super-parents, or brood-stock, of new generations. The assay is based on a key fact that researchers have known for years: myosin, the protein that's the basis of Overturf's new assay, is an important part of muscle in fish and other animals.

Explains Overturf, "Trout that are active users of energy from their feed and are vigorous producers of myosin develop more muscle and grow more quickly. These fish also have less fat. That's because they're converting a large proportion of their feed into muscle, instead of storing it as fat."

But why not simply eyeball trout, using fish size as a potential indicator of continued fast growth? Because the biggest fish aren't necessarily the best. "You can't tell, from their size, which fish are the fastest at converting feed into muscle," says Overturf. But his myosin assay gives users an inside look at this aspect of a trout's genetic make-up. "Superior ability to produce myosin," he says, "is likely a gene-controlled trait."

Overturf's test is the first to use newly emerging real-time PCR technology to assess how actively a rainbow trout's myosin gene is working. Real-time PCR is named for the fact that it accomplishes the many separate, time-consuming steps of conventional PCR all at once, or in what is essentially "real time."

In related work, Overturf has created real-time PCR assays for analyzing various laboratory techniques, scientists isolate DNA from these samples and examine it for the unique patterns of base pairs seen only in family members having the trait.

Before researchers identify the gene responsible for a desired trait, like disease resistance, DNA markers can tell them roughly where the gene is on the chromosome. This is possible because of a genetic process known as recombination. As eggs or sperm develop within a trout's body, the chromosomes within those cells exchange—or recombine—genetic material. If a particular gene is close to a DNA marker, the gene and marker will likely stay together during the recombination process and pass on together from parent to offspring. If each family member with a particular trait also inherits a particular DNA marker, there is a high probability the gene for that trait lies near the marker.

The more DNA markers on a genetic map, the more likely it is that one will be closely linked to the desired trait gene—and the easier it will be for researchers to locate that gene.

"We are using microsatellite markers, which are repetitive stretches of DNA, to create the rainbow trout genetic map," says Rexroad. "This type of marker is easy to use with automated

STEPHEN AUSMUS (K10485-1)

Molecular biologist Caird Rexroad (left) is assisted by fish culturist James Everson while taking tissue samples to be used in developing a genetic map of rainbow trout.

nearly two dozen other gene-derived natural compounds in trout—in addition to myosin. He's done some of that work with his university colleagues.

These assays find, for example, key proteins in trout that indicate a strong immune system, necessary to protect the fish from viruses or bacteria.

The real-time PCR assays are a spinoff of efforts by ARS researchers at Leetown, West Virginia—and by scientists elsewhere in the United States and abroad—to locate and decipher the functions of all the genes in rainbow trout, called the trout genome. (See article above.) Discoveries about the makeup of the trout myosin gene, for instance, provided information Overturf needed to develop the myosin assay.

All the new assays from the Hagerman laboratory are "an immediate, practical application of the rainbow trout genome investigations," says Lewis W. Smith, ARS National Program Leader for Aquaculture Research.

STEPHEN AUSMUS (K10423-1)

Before taking measurements, Ken Overturf (left) and technician Dan Bullock use a tag reader to identify a female brood trout.

The myosin assay was also based, in part, on earlier studies by Overturf and colleague Ronald W. Hardy, director of the Hagerman station. The work yielded new details about the relation of myosin to rainbow trout's prowess in converting feed to muscle. Overturf and Hardy published their findings in *Aquaculture Research*, a British scientific journal.—By **Marcia Wood,** ARS.

This research is part of Aquaculture, an ARS National Program (#106) described on the World Wide Web at www.nps.ars.usda.gov.

Kenneth E. Overturf is in the USDA-ARS Small Grains and Potato Research Unit, c/o Hagerman Fish Culture Experiment Station, 3059F National Fish Hatchery Rd., Hagerman, ID 83332; phone (208) 837-9096, fax (208) 837-6047, e-mail kennetho@uidaho.edu. ★

laboratory equipment so that researchers can rapidly map a trait in a large number of family members."

Rexroad and his colleagues have extracted DNA from each of the 145 families of trout and are adding 500 microsatellite markers they have produced to the genetic map. Rexroad hopes to eventually have 1,000 to 1,500 markers on the map to lay the groundwork for the next phase: functional genomics. Knowing where genes are on the chromosomes is good, but knowing their functions is essential to determining which fish possess specific desirable traits. "In the next year or so, we will be conducting DNA analyses that we hope will determine how these genes function," says Rexroad.

Lending a Helping Hand

In the fishery business, getting fish to marketable size quickly and efficiently makes a big difference in fish producers' financial successes. When finished, this trout map will be used to identify areas on the genome that affect production traits. The objective of the program is to develop a fish that benefits fish producers and consumers. The center is working with the University of Connecticut's Biotechnology Center in Storrs, Connecticut, to find genes that enhance growth rate, increase disease resistance, and improve stress response. It may then be possible to produce transgenic rainbow trout that carry the genes for these qualities and then establish those transgenic founder lines for evaluation of performance.

"By the time this project is finished, our fish will be the most documented crosses of rainbow trout ever," says Hershberger.—By **Sharon Durham,** ARS.

This research is part of Aquaculture, an ARS National Program (#106) described on the World Wide Web at www.nps.ars.usda.gov.

William K. Hershberger and Caird E. Rexroad III are with the USDA-ARS National Center for Cool and Cold Water Aquaculture, 11876 Leetown Rd., Kearneysville, WV 25430; phone (304) 724-8340, fax (304) 725-0351, e-mail bhershbe@ncccwa.ars.usda.gov, crexroad@ncccwa.ars.usda.gov. ★

STEPHEN AUSMUS (K10422-1)

After running real-time PCR assays on genetic material from a rainbow trout, Dan Bullock (left) and Ken Overturf take a moment to study the results.

STEPHEN AUSMUS (K10425-1)

Ken Overturf (left) and hatchery manager Mike Casten determine average sizes and weights of different trout strains.

STEPHEN AUSMUS (K10428-1)

Trout DNA analysis will help researchers produce fish that grow faster, are more disease resistant, and tolerate stress better.